THE REPRODUCTIVE STRUCTURES OF SCHINUS MOLLE (ANACARDIACEAE)

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The *Vergleichende Embryologie der Angiospermen* of Schnarf (1931) has served as the basis and stimulus for very entensive subsequent work in its field. Schnarf accounted for the Anacardiaceae on the basis essentially of a single paper dealing with a single species, being the account of *Rhus Toxicodendron* L. (or *Toxicodendron radicans* O. Kuntze) by Grimm (1912). Subsequent studies of floral morphology and embryology in this family include the following: Juliano (1932) on *Spondias purpurea* L.; Juliano and Cuevas (1932) on *Mangifera indica* L.; Maheshwari (1934) on *Mangifera indica*; Srinivaschar (1940) on *Semecarpus Anacardium* L., *Spondias Mangifera* Willd., and *Anacardium occidentale* L.; Copeland and Doyel[1] (1940) on *Toxicodendron diversilobum* Greene; Sharma (1954) on *Mangifera indica*; Copeland[2] (1955) on *Pistacia chinensis* Bunge; Kelkar (1958a) on *Rhus mysurensis* Heyne; and Kelkar (1958b) on *Lannea coromandelica* (Houtt.) Merrill. The present paper adds *Schinus Molle* L. to the list of species of Anacardiaceae in which the morphology of the reproductive structures is reasonably well known.

Material was collected in the public parks of the City of Sacramento and prepared by routine microtechnical methods (a special technique applied to pollen grains is described below). The results fall short of desired completeness in failing to account for the growth of the pollen tube and for fertilization.

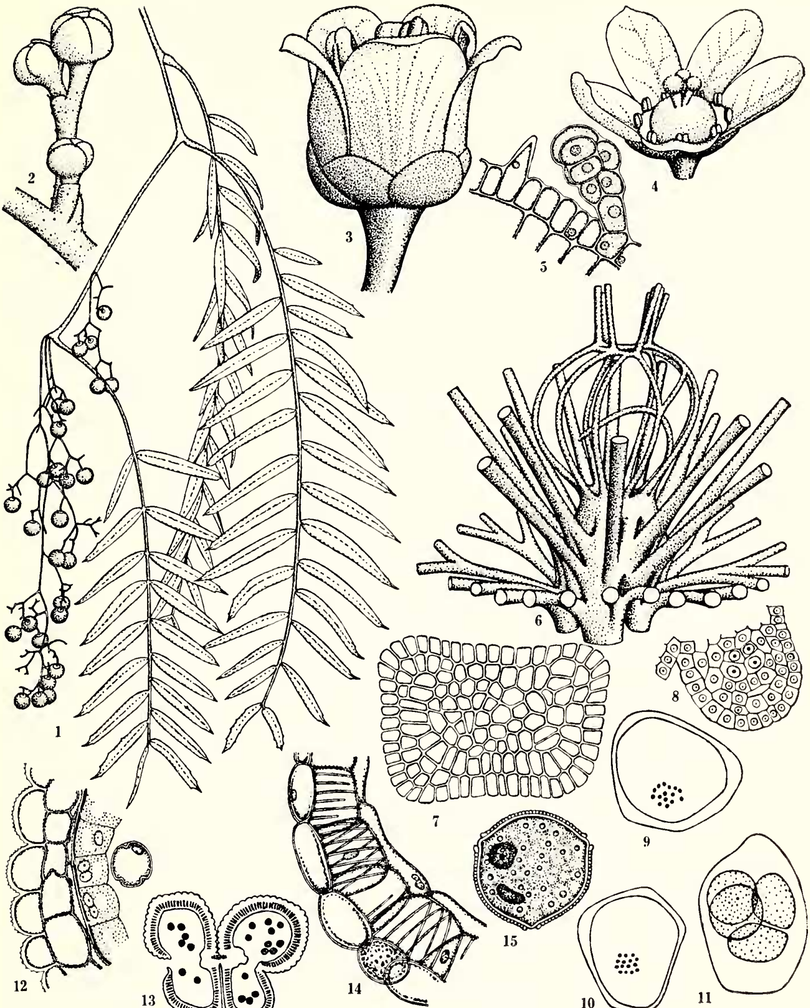
The Tree

Braunton and Davy (1914) tell us that *Schinus Molle* is a native of Peru; that its common name is Peruvian mastic; and that it is very familiar as a cultivated ornamental in southern California, where it has the common name of pepper tree. It is in fact familiar also in northern California, and in many other moderately warm countries.

The tree is fast-growing; of deliquescent form; rarely much more than 10 m. tall; evergreen, with alternate pinnate leaves, the branchlets and leaves often pendant; the small yellow flowers abundant in terminal clusters; dioecious; the female trees producing bunches of purplish-red

[1] Bernice Elva Doyel, afterward Mrs. Harold Strimling, my pupil, associate, and friend, died in Sacramento on February 24, 1954, at the age of 34 years.

[2] It behooves me to acknowledge, and to call to the attention of readers, a ludicrous error in the paper cited. The median stigma of *Pistacia* is said to stand above the large end of the ovary, in which the ovule is attached. Actually it stands above the small end. Referring to a passage in this paper in the second column of page 442, figures 17, 18, 19, 20, and 21 were drawn with the median stigma to the right; my other figures of the gynoecium and its parts were drawn with the median stigma to the left.



FIGS. 1–15. *Schinus Molle:* 1, fruiting twig, × 0.4; 2, cluster of flower buds, × 8; 3, male flower, × 8; 4, female flower, × 8; 5, pubescence of pedicel, × 320; 6, model of the vascular system of a female flower, × 40; 7, cross section of young anther, × 320; 8, cross section of one lobe of an older anther, × 320; 9, 10, heterotypic metaphase in pollen mother cell, × 720; 11, microspore tetrad, × 720; 12, cross section of wall of developing anther, × 320; 13, cross section of mature anther, × 40; 14, portion of cross section of mature anther, × 320; 15, pollen grain, × 720.

drupes about 5 mm. in diameter (fig. 1). It was the odor and taste of these fruits that suggested the common name of pepper tree.

The pepper tree has not the typical reproductive cycle of plants of temperate regions. It produces flowers continually from spring to fall. It responds to the approach of winter by dropping its flower buds, flowers, and young fruits, and by ceasing to grow until the following spring.

In Sacramento, some of the minute axillary buds give rise to branches at about the beginning of the month of March. Each branch bears first two minute alternate scales, these being the covering of the bud. The axis of the branch elongates during a period of from six to eight weeks, producing from three to seven or more foliage leaves. The terminal bud then produces an inflorescence, or else dies: terminal buds do not normally serve as winter buds. Flowers appear first on a particular tree from the middle of May to the middle of June. They are evidently entomophilous, and honey bees have been seen to visit them. Fruits are mature about two months after flowering: the first ones of the year are found at about the beginning of August.

The axis of a flower cluster, continuing that of a branch, grows in indeterminate fashion, bearing a series of alternate scales, but eventually producing a terminal flower. From the axil of each scale grows an axis bearing, ordinarily, two scales and a terminal flower; from the axils of these scales, in basipetal succession, further growth takes place, usually of a single flower, sometimes of a further cluster (fig. 2). The entire cluster of flowers as described is identifiable as a thyrse.

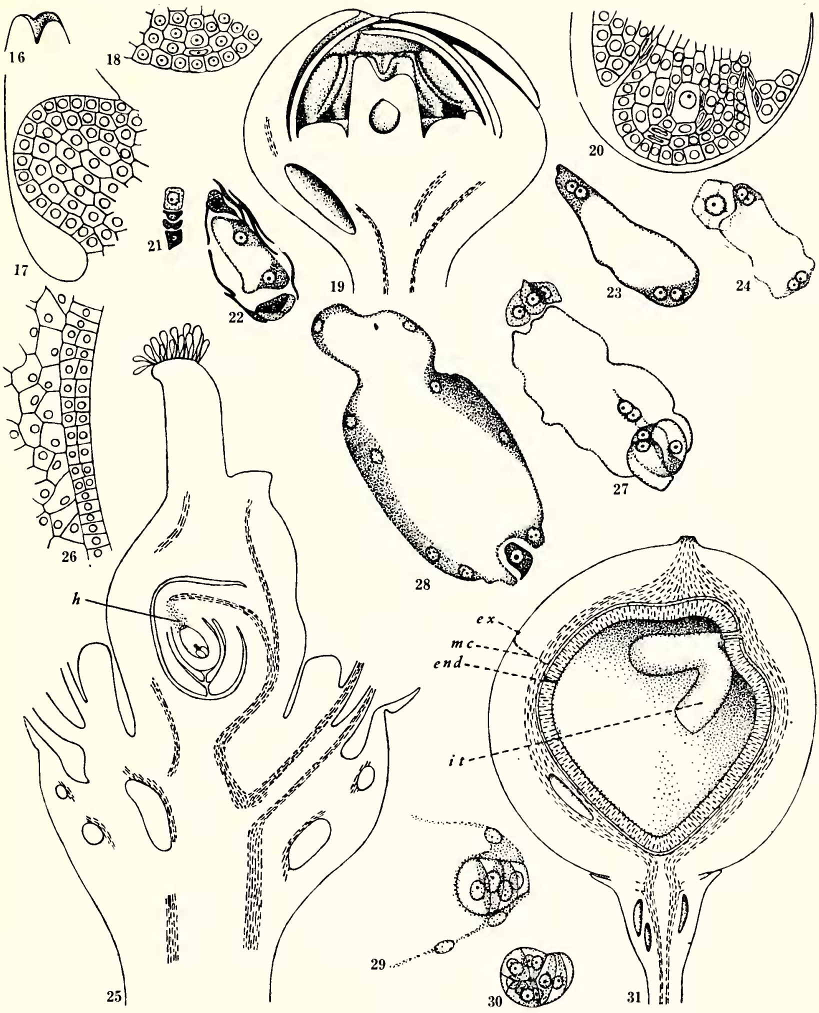
The plant as a whole appears glabrous, but there is a scant pubescence of glandular and simple hairs on the axes and scales of the inflorescence (fig. 5).

Flowers

The male flowers (fig. 3) are slightly larger, and have more erect petals, than the female (fig. 4). Each flower has five sepals, five petals, ten stamens, and a pistil. A prominent disk surrounds the base of the ovary inside the bases of the stamens. A small number of stomata are present in its epidermis. The male flowers have minute and incompletely developed pistils; the female flower bear smaller stamens than the male. The sporogenous cells in the anthers of female flowers undergo abortion at the stage of pollen mother cells.

The mature pistil of the female flower is dorsiventral, but not conspicuously so: in the microtechnical operation of imbedding pistils with the intention of cutting sagittal sections, one makes a considerable number of mistakes. The ovary is of a shape approaching that of an egg laid upon its side: it has a larger and a smaller end; a brief style with a terminal stigma stands in a median position toward the smaller end of the ovary; two lateral styles with their stigmas, not particularly different from the median one, stand above the larger end. As a fairly frequent abnormality, pistils occur with only two styles, one above each end. The ovary contains a single locule almost entirely filled by the single ovule. This is attached to the ovary wall in the median plane of the ovary, at the large end, above the middle (fig. 25).

In the phloem of every vascular bundle, but not extending to the ex-



FIGS. 16–31. *Schinus Molle:* 16, longitudinal section of ovary in young female bud, × 40; 17, beginning of development of ovule, × 320; 18, beginning of periclinal division in the nucellar hypodermis, × 320; 19, longitudinal section of female flower bud, × 40; 20, megaspore mother cell, × 320; 21, tetrad of megaspores, × 320; 22, 2-nucleate embryo sac, × 320; 23, 4-nucleate embryo sac, × 320; 24, an abnormality, an embryo sac developing from the spore next to the chalazal spore, × 320; 25, longitudinal section of female flower with a mature embryo sac, × 40; 26, inner epidermis of ovary of open flower, × 320; 27, mature embryo sac, × 320; 28, undivided zygote in plurinucleate endosperm, × 320; 29, 30, young embryos, × 320; 31, longitudinal section of fruit about one month after anthesis, × 8. *h*, hypostase; *it,* tubular outgrowth of outer integuments; *ex,* exocarp; *mc,* mesocarp; *end,* endocarp.

treme end of the bundle, there is a resin duct, conspicuous under the microscope, being of greater bulk than the vascular tissues. The resin ducts enable one easily to follow the course of the bundles.

About five bundles, arranged in a cylinder, ascend the pedicel and enter the receptacle. In the base of the receptacle they fuse and fork to a certain extent, and send out in radial directions branches which undergo further forking. These are the sepal supply; about five bundles enter each sepal. Above their departure, the stele sends out three successive alternating whorls of five bundles, supplying respectively the petals, the sepalad stamens, and the petalad stamens. In male flowers, the vascular tissue ascending beyond the departure of the stamen traces is scant and quickly fades out. In female flowers (fig. 6), much vascular tissue ascends beyond this point. It is of the form of a truncate cone splitting at the summit to form a whorl of bundles whose typical number is eight. One ascends the small end of the ovary; two ascend each side; at the large end there are typically two ovary wall bundles, and between them, or, often, fused to one of them, the bundle which supplies the ovule. At the summit of the ovary, the ovary wall bundles meet and form a scant network. From this network, branches go up into the styles, each style receiving a median bundle and two laterals.

Male Structures

The stamens are in all respects of the structure typical of flowering plants. Within the four angles of the rudimentary anther (fig. 7), periclinal divisions occur in the hypodermal cells. The progeny of these cells, produced by duly numerous divisions, become differentiated into the following layers of the anther, in order from outward to inward: (a) the endothecium, the hypodermal layer of cells with ribbed walls, whose contraction opens the anther through two longitudinal clefts (figs. 13, 14); (b) two layers of wall cells, of which the outer becomes compressed, and the inner crushed and absorbed (figs. 12, 14); (c) a tapetum of the secretion type, the cells becoming binucleate and then shrivelling and disappearing (fig. 12); and (d) the spore mother cells. In the male flower, meiosis duly takes place within these last. The haploid chromosome number, observed during this process, is 15 (figs. 9, 10). The pollen grains are separated by simultaneous furrowing. The mature pollen grain (fig. 15) has a wall marked by numerous fine pits and three meridional grooves.

The pollen grains contain much stainable material, and it has been difficult to be certain of the number of nuclei. A technique by which they were made visible was as follows: microtome sections, mounted, deparaffined, and hydrated, were exposed for 20 minutes to 1% Na_2CO_3 to which a little NaOH had been added, at 60°C.; stained briefly with methylene blue; and destained in 95% alcohol. Two nuclei are present. The tube nucleus is the larger. It is subglobular and contains a visible nucleolus. The generative nucleus is smaller, fusiform, and heavily staining. It lies within a distinct space, the generative cell

The Ovule

The parts of the flower originate in the bud in acropetal succession. The pistil, in the female bud, originates as a cycle of three knobs, evidently rudimentary carpels, which, as they grow, become coalescent at the base (fig. 16). Somewhat above the bottom of the cavity in the resulting three-pointed cone, the nucellus begins to grow into it from an area below one of the notches between the points (fig. 17). By further growth, the ovary becomes closed above the locule containing the developing ovule (fig. 19). The points above develop, of course, into the styles and stigmas.

The direction of the axis of the nucellus is at first horizontal or slightly upward. It soon bends and grows diagonally downward. At about this time, the inner integument originates as a collar of tissue surrounding the tip of the nucellus and growing forward with it. The hypodermal cells of a small area near the tip of the nucellus begin to undergo periclinal divisions. One of these cells, near the middle of the area, cuts off to the inside a cell, slightly larger than the others, which is to become the megaspore mother cell (fig. 18). By further periclinal divisions, the megaspore mother cell is carried well to the interior of the nucellus (fig. 20); by growth in other parts, an outer integument is produced; both integuments extend beyond the nucellus and close over it, leaving a small micropyle. At the same time that the growth of the ovule produces these changes, it continues to have a bending or coiling effect: the mature ovule is so bent that its axis points to the ovary wall below its insertion (fig. 25).

Meanwhile, the spore mother cell has produced a tetrad of spores (fig. 21), among which the chalazal spore is functional. The nucleus of the functional spore undergoes three divisions (figs. 22, 23). Six of the resulting eight nuclei are set apart in an egg, two synergids, and three antipodal cells, leaving two polar nuclei in the endosperm mother cell (fig. 27). Thus the embryo sac is of the type which Schnarf designated as normal.

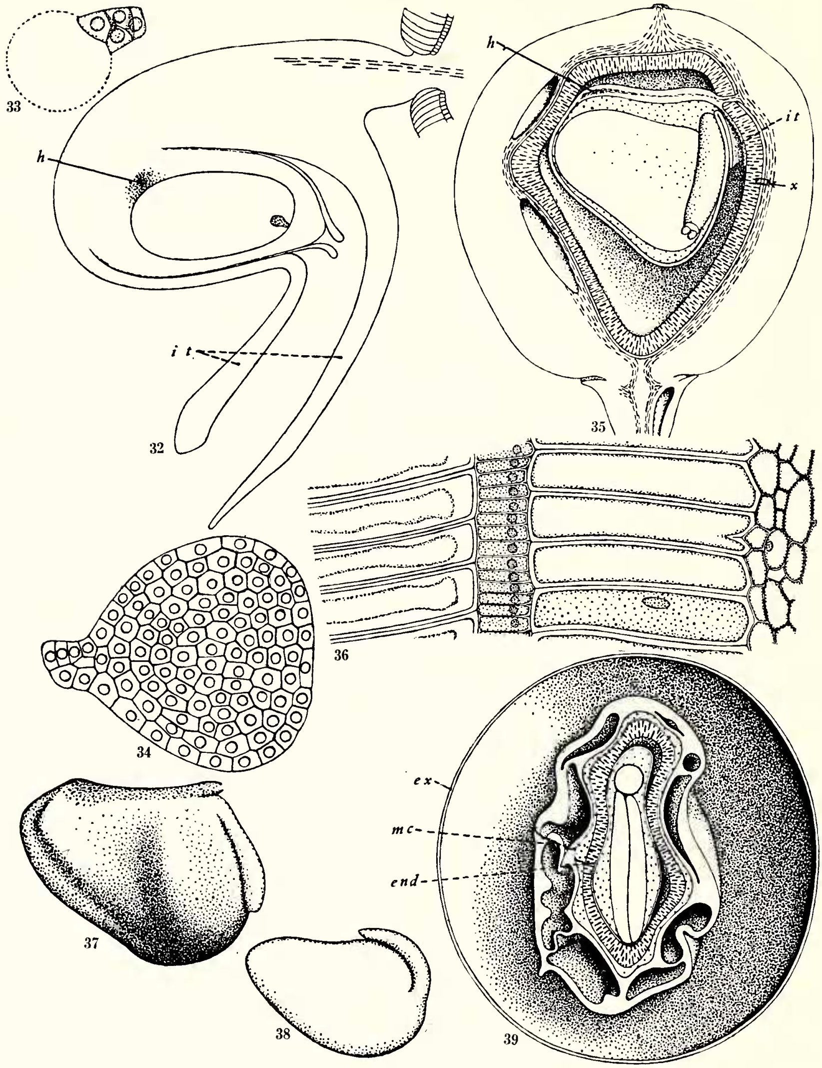
As the embryo sac approaches maturity, there appears in the chalaza, between the end of the bundle traversing the raphe and the antipodal cells, a hypostase (figs. 25, 32, 35), being a body of thick-walled cells of moribund appearance.

A few examples of abnormal development have been noted. An occasional ovule is distorted, twisted otherwise than in the normal downwardly coiled fashion. Sometimes a megaspore other than the chalazal one undergoes development (fig. 24).

Development of Fruit and Seed

The ovary grows during about four weeks after anthesis approximately to the full size of the fruit (fig. 31).

At anthesis, the cells of the inner epidermis of the ovary show periclinal divisions (fig. 26). By these divisions, the epidermis gives rise to three regular layers of cells. The cells of the innermost layer become radially elongate to a length of about 0.2 mm., after which they develop thick



FIGS. 32–39. *Schinus Molle:* 32, longitudinal section of ovule about one month after anthesis, × 40; 33, 34, developing embryos, × 320; 35, longitudinal section of nearly mature fruit, × 8; 36, section of endocarp, area *x* of fig. 35, × 320; 37, mature seed, × 8; 38, mature embryo, × 8; 39, cross section of mature fruit, × 8. ***h,*** hypostase; ***it,*** tubular outgrowth of outer integument; ***ex,*** exocarp; ***mc,*** mesocarp; ***end,*** endocarp.

walls. The middle layer becomes a palisade of thin-walled cells, very much smaller than the preceding. The outer becomes a palisade of thick-walled cells, of about the diameter of those of the inner layer, but only about 0.05 mm. long (fig. 36). These three layers, all derived from the inner epidermis of the ovary, make up the stony endocarp of the fruit. Until about a month after anthesis, it is possible to cut on the microtome paraffin sections of the endocarp; later, the thickness and hardness of the walls make this impossible. The endocarp is deeply impressed by the meridional bundles, with their large resin ducts, in the ovary and fruit. As compared with the more or less egg-shaped ovary and fruit, the endocarp is more strongly compressed in the lateral dimension, being approximately lenticular.

During the first month after anthesis, the ovule grows principally in its micropylar-chalazal dimension. The shape of its axis becomes that of a horizontal U: the end of the nucellus points to an area directly below the insertion of the ovule. The outer integument grows forth as a prominent tube, directed downward in the large end of the ovary at right angles to the rest of the ovule. This structure is presumably without function (figs. 31, 32). It persists in a shrivelled condition in the mature fruit (figs. 35, 37).

The endosperm is of nuclear type. It contains a considerable number of free nuclei before the zygote divides (fig. 28). The nuclei remain free when the embryo is 4-celled (fig. 29); when the embryo is of about a dozen cells (at the stage shown in fig. 30), cellular endosperm has begun to form about it. Further uninucleate cells are cut out, first throughout the surface of the endosperm, and then throughout its interior.

The first division of the zygote has not been seen. It occurs about three weeks after anthesis, and is believed usually to be by an oblique wall. The epibasal cell divides, by walls which are usually oblique, two or three more times. Longitudinal divisions then begin, first in the more distal cells, then in the more proximal, but not usually in the basal cell. Thus when a globular embryo is formed (figs. 33, 34), it has a suspensor which is brief and not sharply set apart, consisting of a basal cell which is not enlarged and of one or two pairs of cells beyond this.

After the fruit and its locule have reached nearly their full size, the seed and embryo enter their phase of most rapid enlargement. The seed fills the locule laterally; its end toward the large end of the fruit becomes vertically elongate; it bulges greatly on the side away from the raphe. The impressions in the endocarp, produced by resin ducts, produce impressions on the sides of the seed.

The vertical elongation of the end of the seed toward the large end of the fruit is accompanied by elongation of the hypocotyl and radicle as a stout column in this end of the seed. The root tip points upward, and remains for a long time near the micropyle, but eventually grows past and curves away from it (fig. 38).

The cotyledons grow forth at right angles to the direction of the hypocotyl. They lie in planes parallel to the median plane of the fruit and seed. The raphe increases only moderately in length; the seed coat and endosperm grow forth beyond its end, and the sharp points of the cotyledons extend beyond the hypostase into the pocket thus formed.

The plumule consists of two small knobs placed decussately with respect to the cotyledons.

After the fruit and seed have reached apparent structural maturity, the mesocarp, containing the bundles and resin ducts, becomes shrivelled. A pigmented filmy exocarp, consisting of the external epidermis of the fruit together with one hypodermal layer, encloses a vacant space. Within this, the mesocarp, of gummy consistency, forms a layer on the surface of the stone. A considerable body of endosperm survives in the mature seed (fig. 39).

Discussion

Here one attempts to characterize the Anacardiaceae by their reproductive structures, microscopic as well as macroscopic. Since the microscopic structures are definitely known only of a moderate number of species, most of which belong to the single tribe Rhoideae, the characterization is acknowledgedly tentative.

The Anacardiaceae bear flowers in cymes which are usually gathered into thyrses. They produce imperfect flowers, some species being dioecious, others polygamous. The imperfect flowers usually appear complete through the presence of rudimentary organs. The flowers are pentamerous, except that in most species the gynoecium is of fewer than five carpels. The ovary or ovaries are superior, surrounded at the base by a prominent disk. (To several of these statements, *Pistacia* offers exceptions, evidently through loss of parts.) In the tribe Rhoideae, the pistil is compound, consisting of one fertile and two sterile carpels, with separate styles and stigmas. There is a single locule. A single ovule is attached basally or else to the wall of the locule, on the side away from the mid-plane of the fertile carpel.

Except in the gynoecium, the receptacular vascular system is undistinguished, a matter of the stele emitting alternating whorls of bundles. The sepalad whorl of stamens is the lower. With the reduction of some of the carpels is associated the fact that the ovary wall bundles are not definitely identifiable as carpel dorsals, carpel laterals, and so forth. When the ovule is basifixed, the bundle supplying it is formed by the anastomosis of several or many bundles running together from all sides. This evidently derived character has been noted outside of tribe Rhoideae in *Mangifera* (Sharma, 1954).

Stamens and pollen show no characters distinguishing Anacardiaceae from flowering plants in general. The four pollen sacs open, by the action of a ribbed endothecium, through two slits. The tapetum is of the secretion type: the cells become and remain binucleate, and remain separate until they are absorbed. The most frequently observed haploid chromosome number is 15; Srinivasachar found 14 in *Spondias*. The pollen grains are separated by simultaneous furrowing. They are binucleate. The exine is in many examples marked by numerous fine pits and by three meridional grooves, and contain two nuclei.

Ovule and seed grow in such fashion as to become more or less strongly apotropously coiled in the median plane of the pistil. Basically, the ovule is bitegmous, but various genera exhibit peculiarities in the development of the integuments, particularly the outer. One suggests for future study the possibility that the obturator, the outgrowth at the base of the funiculus which occurs in various genera but not in *Schinus,* may be morphologically a part of the outer integument.

The ovule is crassinucellate. In the area of the tip of the nucellus, there is a hypodermal archesporium, a layer of cells which undergo periclinal divisions. Only one cell of this layer is functionally archesporial: only one megaspore mother cell is produced (these points, not established in our paper on *Toxicodendron diversiloba,* have been observed in subsequently prepared slides of this species). In the chalaza there is present a hypostase, a body of thick-walled cells of moribund appearance.

The embryo sac is of the type which Schnarf designated as normal. Current usage designates this the *Polygonum* type, a term which fails to express what we believe to be its significance.

The peculiar types of development in the integuments are believed to be associated with the occurence of aporogamy, which is definitely known in *Toxicodendron* and *Pistacia.* Double fertilization is presumed to occur, but has been observed only in *Spondias* (Srinivasachar, 1940).

The fruit grows nearly to its full size before the ovule grows considerably, and the ovule grows considerably before the embryo does so. The occurrence of these three distinguishable cycles of growth was noted in *Mangifera* by Kennard (1955).

The fruit is a drupe. In tribe Rhoideae, the endocarp is derived entirely from the inner epidermis of the ovary: it appears regularly to consist of three definite layers of cells; its stony character is produced by the maturation of one or more of these layers as dense palisades of fiber-like cells. These things appear not to be true of *Mangifera* and *Spondias.*

The endosperm is of nuclear type. It becomes cellular first about the embryo, then throughout its periphery, then in all parts.

The first divisions of the zygote are more or less oblique. A brief suspensor, not definitely set apart, or none, is produced.

Enlargement of the ovule in the course of becoming a seed involves considerable bulging on the side describable as the lower, away from the raphe. The raphe does not elongate considerably, but the enlarging seed coat and endosperm push forth beyond the hypostase which marks the end of the raphe. As the growing seed bulges downward, elongation of the hypocotyl-radicle pushes its proximal end downward, while the root tip remains in the neighborhood of the micropyle. The cotyledons lie in planes parallel to the mid-plane of the gynoecium. They grow forth approximately at right angles to the hypocotyl, and their tips extend beyond the hypostase. A certain amount of endosperm survives in the mature seed.

We will be able to recognize other groups of plants as immediate allies of Anacardiaceae whenever we discover in them assemblages of characters showing a family resemblance to the foregoing. Observations of the same nature as these upon further Anacardiaceae should lead to improvements in the system of the family. In the present state of knowledge, it appears that *Schinus* is relatively primitive, and *Pistacia* relatively advanced.

Summary

Schinus Molle, a South American tree popular as an ornamental in California, where it is called the pepper tree, is dioecious. Flowers of both sexes, produced throughout the summer in thyrses, appear complete, but in each the organs of opposite sex are imperfectly developed.

The male parts exhibit no notable peculiarities: endothecium ribbed; tapetum of secretion type, the cells becoming binucleate; haploid chromosome number 15; pollen grains 3-grooved, binucleate.

The pistil is compound, of one fertile and two sterile carpels, with separate styles and stigmas. The single locule contains a single ovule, attached to the wall on the side opposite the style of the fertile carpel. It is apotropous, bitegmous, crassinucellate. A hypodermal layer of archesporial cells in the distal end of the nucellus produces a single megaspore mother cell. The embryo sac is of normal type.

After anthesis, the ovary grows in about four weeks nearly to the size of the mature fruit; ripening requires another month. The fruit is a drupe whose exocarp, of only two layers of cells, encloses, in an empty space, a gummy mesocarp about a stony endocarp. The endocarp is derived entirely from the inner epidermis of the ovary, and consists of three layers of radially elongate cells, two of them with greatly thickened walls.

Considerable enlargement of the ovule is delayed until the fruit is nearly of its full size, and the embryo grows but little until the ovule is in full course of enlargement. The enlarging ovule is marked by a peculiar tube-like extension of the outer integument. The endosperm is nuclear. The first divisions of the zygote are oblique. The suspensor is brief and not sharply set apart from the embryo proper. In the mature seed the hypocotyl points vertically upward in the end of the seed toward the larger end of the fruit; the cotyledons project at right angles from its lower end; a certain amount of endosperm remains present.

Most of these characters are found, by comparison with those of various other Anacardiaceae, to be characters of family Anacardiaceae, or at least of tribe Rhoideae. Groups other than Anacardiaceae will confidently be recognized as related to this family when sets of characters showing a family similarity to these are observed in them.

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DEVELOPMENT OF THE MEGAGAMETOPHYTE IN LIQUIDAMBAR STYRACIFLUA L.

FRANKLIN F. FLINT

No work has been published on the megagametophyte of *Liquidambar*. Within the family (Hamamelidaceae) only two other genera have been studied in regard to megasporogenesis and megagametogenesis (Flint, 1957, 1957A).

Pistillate floral heads of *Liquidambar styraciflua* were collected in Dinwiddie County, Virginia, from March 28 until July 10, 1953. They were fixed in formalin-aceto-alcohol in the proportions of 90 cc. of 70 percent ethyl alcohol to 5 cc. formalin and 5 cc. of glacial acetic acid. Tertiary butyl alcohol, as used by Johansen (1940), was found most satisfactory for dehydration. The heads were then embedded in tissuemat, sectioned at 10-30 microns, and stained with Harris' Hemotaxylin, Safranin O, and Fast Green FCF.

Around 245 ovules are included in this study. Ovules which showed signs of unusual plasmolysis, nuclear degeneration, or had unusually dark staining cytoplasm were eliminated and are not included in the above count.